

FIG. 1A

PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
Thi	X-X-X-X-X-Thi-X-X-X-X-X-Gly	1.92	1.32	0.54	0.34	0.07	0.04	0.02
Ser-Thi	X-X-X-X-X-Ser-Thi-X-X-X-X-X-Gly	0.11	0.05	0.01	0.00	0.01	0.00	0.00
Threonine* mix	18 phospho-Thi peptide	1.84	1.13	0.40	0.26	0.10	0.07	0.05
Serine* mix	38 phospho-Ser peptide	0.12	0.04	0.02	0.02	0.02	0.01	0.00
Ala-Thi308-P	Ile-Lys-Asp-Gly-Ala-Thi-Met-Lys-Thi-Phe-Gly-Gly-Thi-Pro (SEQ ID NO:1)	1.18	0.65	0.24	0.13	0.03	0.01	0.00
APP1-Thi868-P	Asp-Ala-Ala-Val-Thi-Pro-Lys-Lys-Ala-His-Leu-Ser-Lys-Gly (SEQ ID NO:2)	0.14	0.03	0.01	0.01	0.01	0.01	0.00
C3-P	Asp-Thi-Glu-Ile-Lys-Ala-Ser-Thi-Phe-Val-Gly-Thi-Pro-Phe-Gly (SEQ ID NO:3)	1.71	1.13	0.39	0.22	0.03	0.02	0.02
CAR-Thi167-P	His-Glu-Val-Val-Thi-Ala-Tyr-Tyr-Ala-Gly (SEQ ID NO:4)	1.77	1.15	0.41	0.27	0.08	0.03	0.01
CAMIV-Thi186-P	His-Glu-Val-Ile-Met-Lys-Thi-Val-Gly-Gly (SEQ ID NO:5)	1.79	1.36	0.63	0.40	0.09	0.05	0.01
CDCs-Thi167-P	Ile-Pro-Ile-Ala-Val-Thi-Thi-His-Glu-Val-Val-Thi-Leu-Gly (SEQ ID NO:6)	1.02	0.56	0.14	0.08	0.03	0.01	0.01
CDKc-Thi159-P	Gly-Val-Pro-Val-Ala-Thi-Thi-His-Glu-Val-Val-Thi-Leu-Gly (SEQ ID NO:7)	1.88	1.79	0.51	0.44	0.08	0.04	0.02
P70S8K-Thi389-P	Asp-Glu-Val-Phe-Leu-Gly-Phe-Thi-Tyr-Val-Ala-Pro-Lys-Lys-Gly (SEQ ID NO:8)	1.89	1.44	0.62	0.39	0.06	0.04	0.01
PKCalpha-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thi-Thi-Ala-Thi-Phe-Gly (SEQ ID NO:9)	1.82	1.63	0.84	0.58	0.18	0.08	0.02
BRQ-P	Asp-His-Thi-Gly-Phe-Leu-Thi-Glu-Tyr-Val-Ala-Thi-Ala-Tyr-Gly (SEQ ID NO:10)	1.56	1.18	0.51	0.30	0.07	0.04	0.02
Mye Ser58/62-P	Glu-Leu-Leu-Pro-Thi-Pro-Pro-Leu-Ser-Pro-Ser-Ala-Ala-Ser-Gly (SEQ ID NO:11)	0.11	0.05	0.03	0.02	0.02	0.02	0.02
P38-2P	Leu-Ala-Ser-His-Thi-Asp-Ala-Glu-Met-Thi-Gly-Tyr-Val-Ala-Thi-Ala-Gly (SEQ ID NO:12)	0.64	0.30	0.08	0.08	0.04	0.04	0.02
INIV-2P	Ser-Phe-Met-Met-Thi-Pro-Tyr-Val-Val-Thi-Ala-Tyr-Tyr-Ala-Gly (SEQ ID NO:13)	1.88	0.44	0.12	0.07	0.03	0.02	0.02

(SEQ ID NO:13)

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FIG. 1B

PEPTIDE SEQUENCE	phospho-Thr Reactivity
XXXXXXS*XXXXXX	—
XXXXY*XXXX	—
XXXXXPXS*/T*PXR/KXXX (SEQ ID NO:14)	++
XXXXRSXS*XPXXXX (SEQ ID NO:15)	—
XXXXRSXSXPXXXX (SEQ ID NO:16)	—
XXXXXPXS*/T*PXXXXX (SEQ ID NO:17)	++
XXXXXPXS/TPXXXXX (SEQ ID NO:18)	—
XXXXXT*XXXXXX	+++
XXXXXXS/TXXXXXX	—
21 phospho-Thr peptides mixture	+++
38 phospho-Ser peptides mixture	—
30 phospho-Tyr peptides mixture	—
NEB LIBRARY	
X-X-X-X-D/E-X-X-S*-T*-X-X-X-X-X-C (SEQ ID NO:19)	+++
X-X-X-X-X-X-S*/T*-D/E-D/E-D/E-X-X-X (SEQ ID NO:20)	++
X-X-X-X-F-X-X-F-S*/T*-F/Y-X-X-X-X-C (SEQ ID NO:21)	+++
X-X-X-X-R/K-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:22)	+++
X-X-X-R/K-X-X-S*/T*-X-X-X-X-X-X-C (SEQ ID NO:23)	+++
X-X-X-X-X-X-S*/T*-F/I/M-X-X-X-X-X-C (SEQ ID NO:24)	+++
X-X-X-X-X-X-S*/T*-F/I-X-X-X-X-X-C (SEQ ID NO:25)	+++
X-X-X-X-X-X-S*/T-P-X-X-X-X-X-X-C (SEQ ID NO:26)	++
X-X-X-X-X-T*-X-X-X-X-X-X-C	+++
X-X-X-X-X-P-X-S*/T*-P-X-X-X-X-X-C (SEQ ID NO:27)	++
X-X-X-X-X-X-S/T-X-X-X-X-X-X-C (SEQ ID NO:28)	—
X-X-X-X-X-P-X-S*/T*-P-X-R/K-X-X-X-C (SEQ ID NO:29)	++
ANTIBODY REACTIVITY	
+++ very strong	ELISA O.D. > 2
++ strong	1 - 2
+ weak	0.2 - 1
- very little	< 0.2

004250 + 495550

004250-19E5E560

FIG. 1C

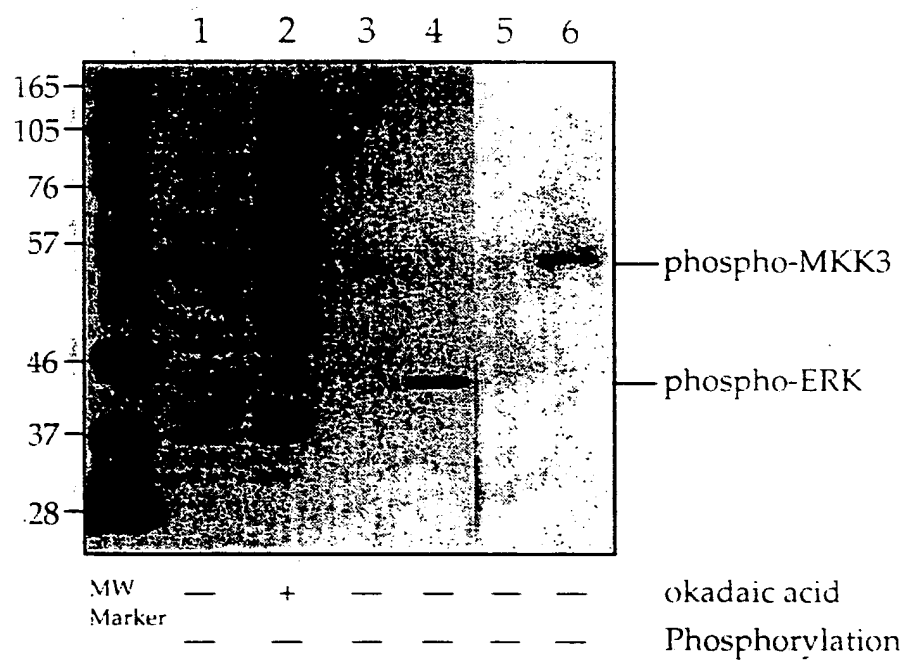


FIG. 1D

Fixed Amino Acid	-5-4-3-2-1 X X X X X Ser* / Thr* X X X X X Fixed AA position relative to phospho-Ser* / Thr*									
	-4-3-2-1				S* / T*	+1+2+3				
	-4	-3	-2	-1		+1	+2	+3		
Ala	++	++	+++	+++		+++	+++	++		
Cys	+++	+++	+++	+++		+++	+++	++		
Asp	++	++	++	+++		+++	+++	++		
Glu	++	++	++	+++		+++	+++	++		
Phe	++	++	++	+++		+++	+++	++		
Gly	++	++	++	++		+++	+++	++		
His	++	++	++	+++		+++	+++	++		
Ile	++	++	++	+++		+++	+++	++		
Lys	++	++	++	+++		+++	+++	++		
Leu	++	++	++	+++		+++	+++	++		
Met	++	++	++	+++		+++	+++	++		
Asn	++	+++	++	+++		+++	+++	++		
Pro	++	++	++	+++		—	+++	++		
Gln	++	++	++	+++		+++	+++	++		
Arg	++	++	++	+++		+++	+++	++		
Ser	++	++	++	++		+++	+++	++		
Thr	++	++	++	+++		+++	+++	++		
Val	++	++	++	+++		+++	+++	++		
Trp	++	++	++	+++		+++	+++	++		
Tyr	++	++	++	+++		++	+++	++		

FIG. 2A

PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	ANTIBODY DILUTIONS	1.00E+04	5.00E+04	1.00E+05	5.00E+05	1.00E+06
PKSPSP	X-X-X-X-X-Pro-X-Ser-Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO: 27)	1.82	1.97	1.74	1.40	0.70	0.35	0.08	
Threonine mix	18 phospho-Thr peptide mix	1.87	1.37	0.87	0.36	0.13	0.07	0.05	
Ser/Thr	X-X-X-X-X-Ser/Thr-X-X-X-X-X-X-Cys (SEQ ID NO: 28)	0.14	0.03	0.01	0.00	0.00	0.00	0.00	0.00
RB Thr373-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Alg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO: 30)	2.07	2.17	1.70	1.20	0.48	0.18	0.03	
MIK33-Thr-P	Ser-Val-Ala-Lys-Thr-Met-Asp-Ala-Gly-Cys (SEQ ID NO: 31)	0.06	0.04	0.01	0.00	0.00	0.00	0.00	0.00
PKCαphs-P	Lys-Glu-His-Met-Met-Asp-Gly-Val-Thr-Thr-Arg-Thr-Phe-Cys (SEQ ID NO: 9)	0.05	0.02	0.01	0.00	0.01	0.00	0.00	0.00
P70 S6K-Thr368	Asn-Gln-Val-Phe-Leu-Gly-Phe-Thr-Tyr-Val-Ala-Pro-Lys-Lys-Cys (SEQ ID NO: 8)	0.11	0.05	0.01	0.00	0.01	0.00	0.00	0.00
cdk4-Thr172-P	Arg-Ile-Tyr-Ser-Tyr-Gln-Met-Ala-Leu-Thr-Pro-Val-Val-Val-Lys-Cys (SEQ ID NO: 32)	2.07	2.21	2.01	1.55	0.89	0.31	0.07	

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FIG. 2B

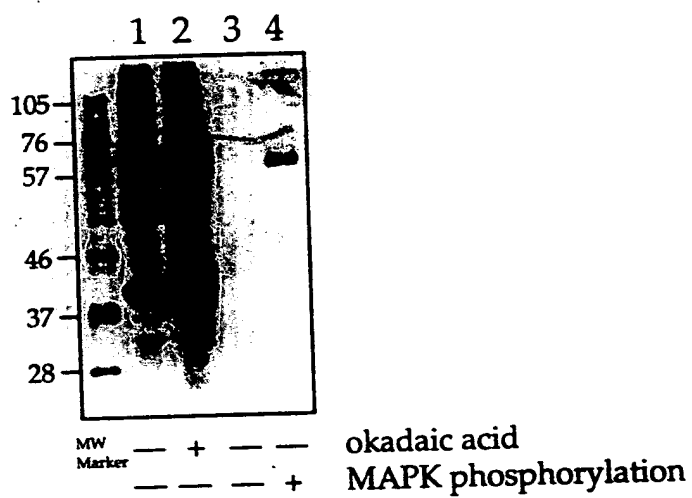
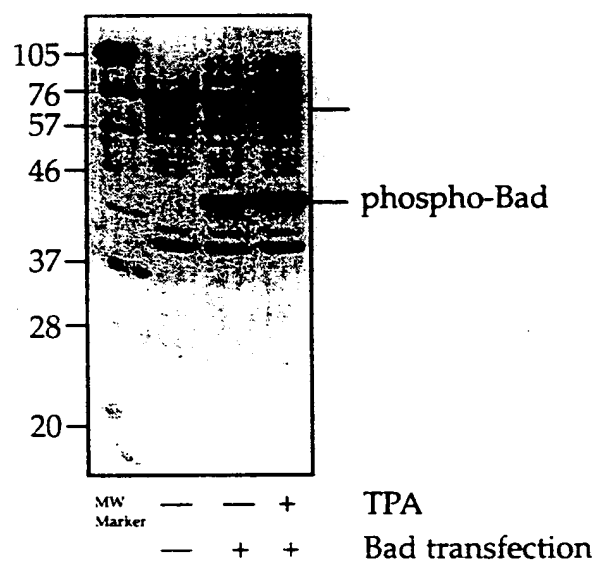


FIG. 3A

				ANTIBODY DILUTIONS				
PEPTIDE	SEQUENCE	1.00E+03	5.00E+03	1.00E+04	5.00E+04	1.00E+05	5.00E+05	
14-3-3 BM-P	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-Cys (SEQ ID NO: 33)	2.41	2.15	1.49	1.15	0.44	0.25	
14-3-3 BM	X-X-X-X-Arg-Ser-X-Ser-X-Pro-X-X-X-Cys (SEQ ID NO: 34)	0.07	0.03	0.02	0.03	0.02	0.03	
CDC25-Ser216-P	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys (SEQ ID NO: 35)	2.35	2.08	1.49	1.05	0.33	0.18	
CDC25-Ser216	Gly-Leu-Tyr-Arg-Ser-Pro-Ser-Met-Pro-Glu-Asn-Leu-Asn-Arg-Cys (SEQ ID NO: 36)	0.05	0.02	0.03	0.03	0.04	0.03	
Bad-Ser112-P	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys (SEQ ID NO: 37)	1.59	0.43	0.10	0.03	0.01	0.00	
Bad-Ser112	Thr-Arg-Ser-Arg-His-Ser-Ser-Tyr-Pro-Ala-Gly-Thr-Glu-Glu-Cys (SEQ ID NO: 38)	0.00	0.00	0.00	0.00	0.00	0.00	
Bad-Ser136	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys (SEQ ID NO: 39)	0.03	0.00	0.00	0.00	0.00	0.00	
Bad-Ser136-P	Phe-Arg-Gly-Arg-Ser-Arg-Ser-Ala-Pro-Pro-Asn-Leu-Trp-Ala-Cys (SEQ ID NO: 40)	3.25	1.86	0.73	0.51	0.07	0.03	

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

FIG. 3B



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FIG. 4A

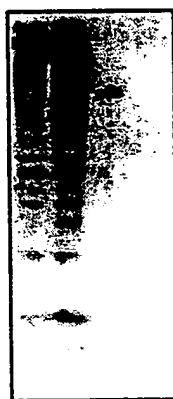
PEPTIDE	SEQUENCE	MONOCLONAL ANTIBODIES	
		6B6	6A9
Ser/Thr/Pro-P	X-X-X-X-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO. 26)	1.774	0.731
ProXSer/Thr/Pro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO. 27)	0.924	0.766
ProXSer/Thr/Pro-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-X-X-X-X-Cys (SEQ ID NO. 41)	0.02	0.063
ProXSer/Thr/ProXArg-P	X-X-X-X-X-Pro-X-Ser/Thr-Pro-X-Arg-Lys-X-X-X-Cys (SEQ ID NO. 42)	1.955	1.275
Thr-P	X-X-X-X-X-X-Thr-X-X-X-X-X-X-Cys	0	--
Ser-P	X-X-X-X-X-X-Ser-X-X-X-X-X-X-Cys	0.031	0.088
Ser/Thr	X-X-X-X-X-X-Ser/Thr-X-X-X-X-X-Cys	0.021	0.066
Tyr-P	X-X-X-X-X-X-Tyr-X-X-X-X-X-X-Cys	0.023	0.072
Rb (Ser795)-P	Ser-Pro-Tyr-Lys-Phe-Pro-Ser-Ser-Pro-Leu-Arg-Ile-Pro-Gly-Cys (SEQ ID NO. 43)	0.032	0.124
Rb (Thr373)-P	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO. 30)	3.336	3.503
Rb (Thr373)	Val-Ile-Pro-Pro-His-Thr-Pro-Val-Arg-Thr-Val-Met-Asn-Thr-Cys (SEQ ID NO. 44)	0.02	0.073

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FIG. 4B

6B8

1 2 3 4



okadaic acid	-	+	-	-
phosphorylated RB	-	-	+	-
RB	-	-	-	+

5A9

5 6



okadaic acid	-	+
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004420-436560

FIG. 5A

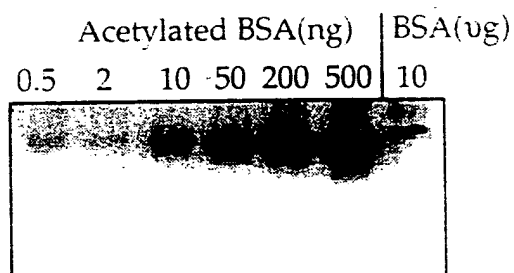


FIG. 5B



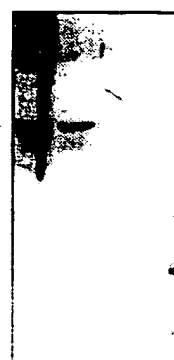
1 2 3 4

FIG. 5C



5 6 7 8

FIG. 5D



9 10 11 12

Phospho-Akt Substrate Antibody

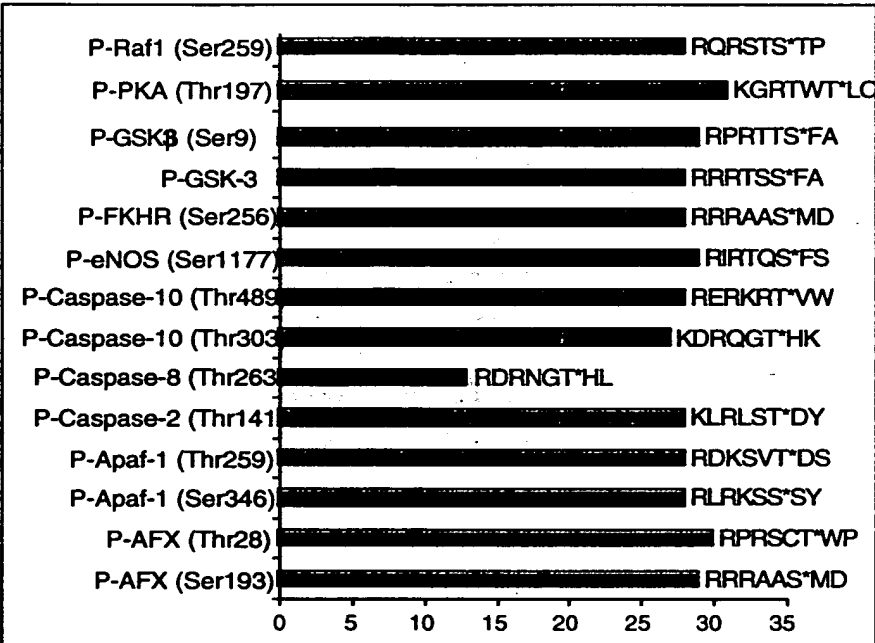


Figure 6: Signal to noise ratio of ELISA readings using Phospho-Akt Substrate Antibody with phospho-peptides of Akt substrates vs. non-phospho-peptides of Akt substrates.



- +
calyculin A

Figure 7: Western analysis of calyculin A-treated A431 cells using Phospho-Akt Substrate Antibody.

Phospho-PKA Substrate Antibody

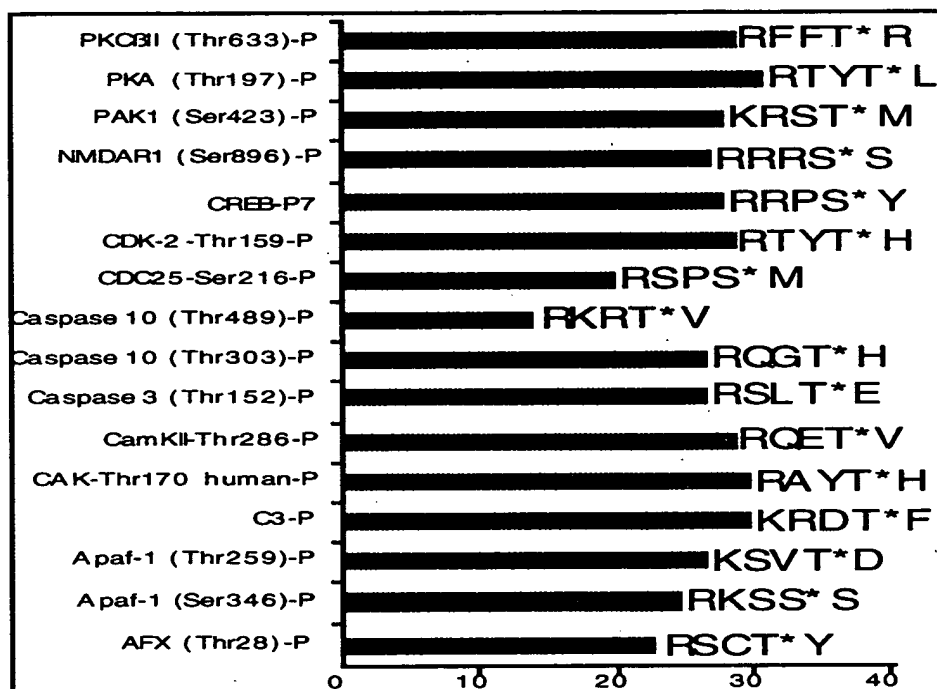


Figure 8: Signal to noise ratio of ELISA reading using phospho-PKA substrates antibody against peptides have Arginine or Lysine at -3 position.

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Phospho-PKA Substrate Antibody

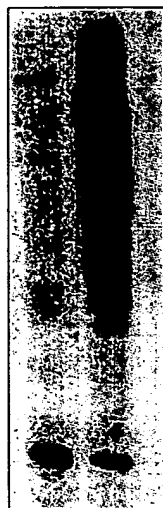


Figure 9: Western analysis of calyculin A-treated A431 cells using Phospho-PKA Substrate Antibody.

- + calyculin A

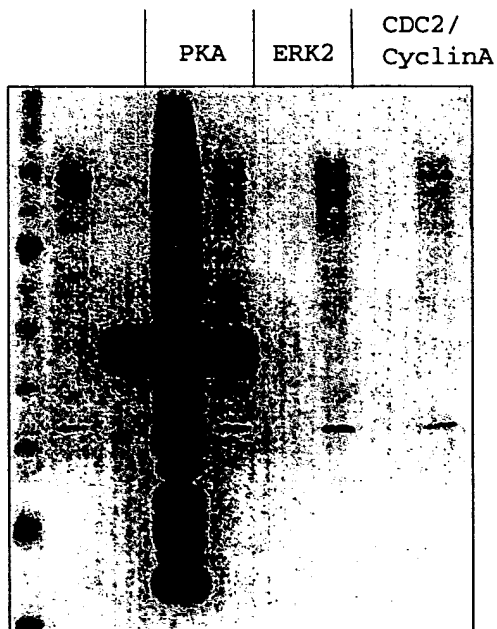


Figure 10: Western analysis of A431 cell extracts phosphorylated by protein kinase A, ERK2 or CDC2/cyclinA in vitro using Phospho-PKA Substrate Antibody.

+	-	+	+	-	+	-	+	Cell Extracts
-	-	-	+	-	-	-	-	PKI

Phospho-Serine/Threonine Phenylalanine Antibody

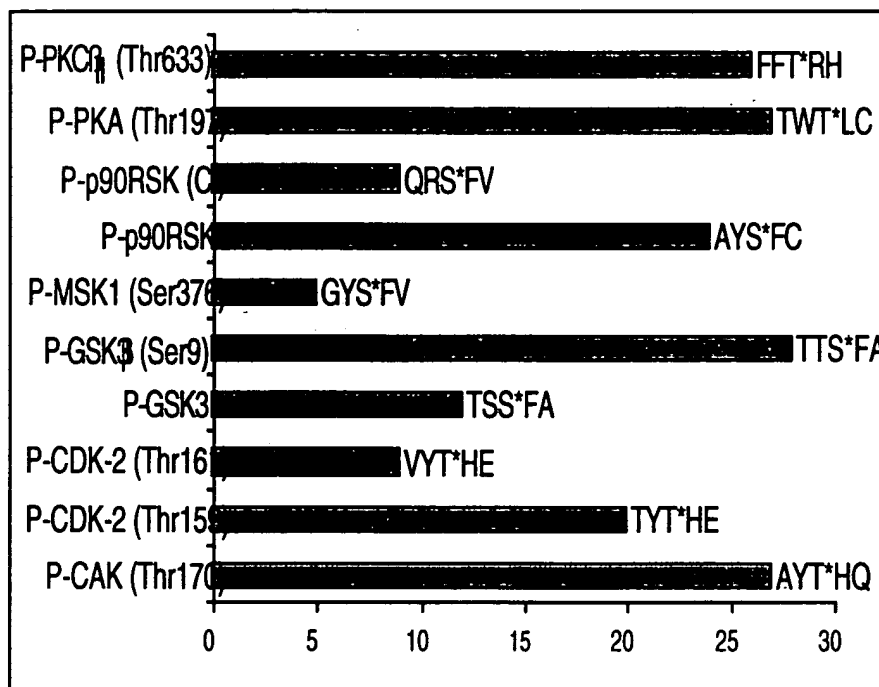


Figure 11: Signal to noise ratio of ELISA reading using phospho-Serine/threonine phenylalanine antibody against the peptides surrounded by phenylalanine, tyrosine or tryptophan.



Figure 12: Western analysis of calyculin A-treated A431 cells using phospho-Serine/phenylalanine substates antibody.

- +
calyculin A